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Research Note

Induction of Increased Benomyl Tolerance in *Verticillium lecanii*, a Fungus Antagonistic to Plant-Parasitic Nematodes[1]

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ABSTRACT: Following exposure of *Verticillium lecanii* to ultraviolet radiation, 4 mutants were induced that exhibited greater tolerance to the fungicide benomyl than did the wild type. Colonies of the wild type strain increased in diameter at a rate of approximately 4–8 mm per week on 100 μg benomyl per ml potato dextrose agar; the mutants increased in diameter approximately 10–17 mm per week on the same agar.

KEY WORDS: *Verticillium, Heterodera,* soybean cyst nematode, nematode, biological control, genetic manipulation, mutation.

Verticillium lecanii (A. Zimmermann) Viégas has been studied as a control agent for insects and fungi (Hussey, 1984; Harper and Huang, 1986; Uma and Taylor, 1987; Heintz and Blaich, 1990), and strains have been commercially produced as biocontrol agents for aphids and whiteflies. Research on the fungus as a control agent for plant-parasitic nematodes has included isolation of *V. lecanii* from nematodes in the field, and laboratory experiments to determine whether the fungus affects nematode viability (Hänssler and Hermanns, 1981; Gintis et al., 1983; Rodríguez-Kábana and Morgan-Jones, 1988; Hänssler, 1990; Meyer et al., 1990). Four strains of *V. lecanii* were tested in petri dish cultures for antagonism to eggs of the soybean cyst nematode, *Heterodera glycines* Ichinohe (Meyer et al., 1990). Strain 58909 from the American Type Culture Collection caused a significant decrease in numbers of viable soybean cyst nematode (SCN) eggs.

The antagonistic strain 58909 was studied for tolerance to the fungicide benomyl (Meyer et al., 1991). Experiments were then conducted to determine whether benomyl tolerance could be increased by exposure to ultraviolet light. Previous mutagenesis studies on *V. lecanii* have produced changes in spore density and spore release, alterations in enzyme activity, strains with altered pigmentation on the undersides of colonies, and auxotrophic mutants (Jackson, 1984; Heale, 1987). There were several reasons for attempting to induce mutants with resistance or increased tolerance to benomyl. Benomyl can be deleterious to some strains of *V. lecanii* applied for biological control of insects in the greenhouse (Gardner et al., 1984; Hassan and Oomen, 1985). Benomyl is registered for use on soybean and other field crops, and may be applied as part of an integrated pest management (IPM) program. If *V. lecanii* was to be used as a biocontrol fungus in an IPM system, a strain with high benomyl tolerance might survive more readily than a fungus with no tolerance. In addition, benomyl could be incorporated into the fungus delivery system to discourage other organisms from growing on nutrients applied with the control fungus. A further potential benefit of increased benomyl tolerance is that it may serve as a marker to aid in identification of biocontrol strains. Another reason for employing benomyl is that some fungus mutants with increased benomyl tolerance have greater biocontrol capacity than the wild type strains, even when benomyl has not been applied to a crop (Papavizas, 1985).

Studies were conducted on nematode-antagonistic fungi to determine if benomyl tolerance or resistance would improve biocontrol ability (Gaspard and Mankau, 1985; Gaspard, 1986). Conidia of the fungi *Paecilomyces lilacinus* and *Verticillium chlamydosporium* were irradiated with ultraviolet light, and biotypes with resistance or tolerance to benomyl were induced. The isolates and the wild types were able to parasitize eggs of *Meloidogyne* spp., but neither the wild type nor the mutant strains of *P. lilacinus* significantly reduced root knot nematode egg numbers on tomato. Induction of benomyl resistant mutants from wild type strains of fungi that significantly reduce nematode populations may result in more successful biocontrol agents.

To induce mutants of *V. lecanii,* suspensions

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of conidia were made in sterile distilled water, and plated onto either potato dextrose agar (PDA) or PDA + benomyl. Benomyl (Benlate 50 Wettable Powder, E. I. du Pont de Nemours & Co., Wilmington, Delaware) amended plates contained 100 μg benomyl/ml PDA. This concentration of benomyl was selected because the wild type strain was not able to grow as quickly as on unamended PDA, but the benomyl level did not severely inhibit the growth of the fungus. The mutants could be selected by choosing colonies that grew the most quickly on the agar. Two hundred one petri dishes were each inoculated with approximately 200 conidia. Six petri dishes contained PDA; 195 petri dishes contained PDA + benomyl. Conidia in 3 petri dishes of PDA and 3 dishes of PDA + benomyl were not UV-irradiated. The rest of the conidia were exposed for 40 sec to ultraviolet radiation from a General Electric G30T8 30-watt germicidal bulb. If UV-irradiated spores of *Verticillium dahliae* and *Verticillium albo-atrum* are incubated in the light, fewer mutants are produced because photoreactivating enzymes repair DNA damage from ultraviolet light (Puhalla, 1973). Consequently, petri dishes containing irradiated conidia of *V. lecanii* were placed in boxes or were wrapped in foil to prevent exposure to light. Counts of viable colonies indicated that the survival rate after 40-sec irradiation was approximately 39%. After an incubation period of 8 days at 25°C, colony diameters were compared. Eight to 14 days after irradiation, 11 colonies on irradiated plates were greater in diameter than the other colonies growing on PDA + benomyl. To minimize genetic variability, 3–6 single spore isolates were made of each of the 11 colonies.

To determine whether the isolated colonies were mutants with increased tolerance to benomyl, plugs 9 mm in diameter were made from each single spore isolate and were inoculated onto PDA and PDA + 100 μg benomyl/ml medium. Colony growth was measured at 1 and 2 wk after inoculation. Seven of the strains that had originally appeared to grow more rapidly on benomyl-amended agar did not do so when tested in a quantifiable experiment. Those 7 colonies may have had large diameters on PDA + benomyl following irradiation because they grew from conidia that germinated quickly after inoculation, or because the large colonies formed when smaller colonies became confluent. Only 4 of the 11 strains had greater growth rates on the benomyl-amended agar than the wild type strain: strains 1, 2, 9, and 10. On PDA + benomyl, colonies of the wild type strain increased in diameter ca. 4 mm the first week and ca. 8 mm the second week. The mutants increased about 11–17 mm the first week and approximately 10–17 mm the second week. However, these strains did not grow as quickly as the wild type strain on the unamended agar. On PDA, colony diameters of the wild type strain increased approximately 18 mm the first week and 24 mm the second week, whereas mutants increased in diameter ca. 12–20 mm the first week and ca. 13–20 mm the second week.

These results show clearly that strains of *V. lecanii* with increased benomyl tolerance can be induced with exposure to ultraviolet light. In greenhouse experiments (Meyer, 1990; Meyer and Huettel, 1991), the wild type strain and a tested mutant both reduced nematode populations in the soil, but the mutant was more efficacious than the wild type at low levels of application. This result occurred in the absence of benomyl. A culture of each of the four strains has been deposited at the Agricultural Research Service Culture Collection (NRRL). The cultures have been assigned the strain numbers NRRL 18725, 18726, 18727, and 18728.

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Research Note

Occurrence of Larval *Contracaecum* sp. (Ascaridida: Anisakidae) in Rio Grande Lesser Sirens, *Siren intermedia texana* (Amphibia: Caudata), from South Texas

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ABSTRACT: Unencapsulated third-stage larval anisakid nematodes, *Contracaecum* sp. Railliet and Henry, 1912, were recovered from the coelomic cavity of all of 8 Rio Grande lesser sirens, *Siren intermedia texana* Goin, 1957, from southern Texas. Mean intensity was 2.1 (range 1–5) worms per host. This is the first report of larval *Contracaecum* sp. infecting a caudate amphibian.

KEY WORDS: Anisakidae, Ascaridida, Nematoda, *Contracaecum* sp., Caudata, *Siren intermedia texana,* Sirenidae.

The Rio Grande lesser siren, *Siren intermedia texana* Goin, 1957, is a large eellike salamander that ranges from the lower Rio Grande Valley of Texas to Tamaulipas, Mexico (Martof, 1973; Dixon, 1987). The species inhabits a wide variety of aquatic sites. In Texas, *S. i. texana* is considered an endangered taxon and is afforded protection by the Texas Parks and Wildlife Department.

Although a great deal of information is available on endoparasites of conspecific western lesser sirens, *S. i. nettingi* Goin, 1942 (Nickol, 1972; Dunagan and Miller, 1973; Dyer, 1973; Brooks and Buckner, 1976; Brooks, 1978; Buckner and Nickol, 1979), nothing has been published on parasites of *S. i. texana.* During a morphometric study of *S. i. texana* (McDaniel, 1969), several